acetone; yield 1.3 g. (90%); m.p. 171–173°; $[\alpha]^{28}D$ +17.9° (*c* 3.46, in pyridine).

Anal. Calcd. for $C_{23}H_{28}O_8N_6$: C, 53.5; H, 5.5; N, 16.3. Found: C, 53.4; H, 5.4; N, 16.2.

Carbobenzoxynitro-L-arginyl-L-tryptophan.—Carbobenzoxynitro-L-arginyl-L-tryptophan methyl ester (1.6 g.) was suspended in 0.5 *N* sodium hydroxide (10 ml.) and the mixture was shaken for 1 hour. The product was isolated in the usual manner and recrystallized from 50% aqueous ethanol; yield 1.36 g. (88%); m.p. 202–203°; $[\alpha]^{30}D$ +20.8° (*c* 1.45, in pyridine).

Anal. Calcd. for $C_{23}H_{29}O_7N_7$: C, 55.7; H, 5.4; N, 18.2. Found: C, 55.6; H, 5.7; N, 18.5.

Carbobenzoxynitro-L-arginyl-L-glutamic Acid.—Carbobenzoxynitro-L-arginyl-L-glutamic acid diethyl ester (1.1 g.) was suspended in 0.5 *N* sodium hydroxide (12 ml.) and the mixture was shaken for 1 hour. The product was isolated in the usual manner and recrystallized from 50% aqueous ethanol; yield 0.84 g. (87%); m.p. 224–225° (lit.[3] m.p. 211–212°); $[\alpha]^{27}D$ 0.0° (*c* 0.95, in pyridine).

Anal. Calcd. for $C_{19}H_{26}O_9N_6$: C, 47.3; H, 5.4; N, 17.4. Found: C, 47.6; H, 5.5; N, 17.5.

L-Arginylglycine Acetate, Acetic Acid Solvate.—Carbobenzoxynitro-L-arginylglycine (1 g.) was dissolved in methanol containing 10% of glacial acetic acid (25 ml.). Palladium catalyst[19] was added and the mixture was shaken in a stream of hydrogen for 12 hours. The catalyst was removed by filtration and the solvent was evaporated *in vacuo*. The ensuing sirup was layered with acetone and kept at room temperature until crystallization was complete. The material was recrystallized from methanol-acetic acid; yield 0.74 g. (88%); m.p. 167–169°; $[\alpha]^{28}D$ +38.9° (*c* 5.75, in water).

Anal. Calcd. for $C_{10}H_{21}O_5N_5(CH_3COOH)$: C, 41.0; H, 7.2; N, 19.9; acetyl, 34.2. Found: C, 41.4; H, 7.0; N, 20.1; acetyl, 32.2.

L-Arginyl-L-alanine Acetate.—Carbobenzoxynitro-L-arginyl-L-alanine (1.0 g.) was hydrogenated in methanol containing 10% of acetic acid (25 ml.) in the manner described above. The sirup resulting from the evaporation of the solvents crystallized on standing. The material was recrystallized twice from aqueous ethanol; yield 0.6 g. (83%); m.p. 173–174°; $[\alpha]^{32}D$ +9.7° (*c* 2.38, in water).

Anal. Calcd. for $C_{11}H_{23}O_5N_5$: C, 43.3; H, 7.6; N, 22.9. Found: C, 42.7; H, 7.1; N, 23.1.

L-Arginyl-L-leucine Acetate.—Carbobenzoxynitro-L-arginyl-L-leucine (1.3 g.) was hydrogenated in methanol containing 10% of acetic acid (25 ml.) in the manner described above. The dipeptide crystallized during the hydrogenation and was redissolved by the addition of glacial acetic acid (15 ml.). The crystals resulting on evaporation of the solvents were recrystallized from methanol-acetic acid; yield 0.85 g. (89%); m.p. 205–206° (lit.[7] m.p. 207–208°); $[\alpha]^{28}D$ +9.6° (*c* 1.35, in water).

Anal. Calcd. for $C_{14}H_{29}O_5N_6$: C, 48.4; H, 8.4; N, 20.2. Found: C, 48.5; H, 8.2; N, 20.0.

L-Arginyl-L-phenylalanine Acetate.—Carbobenzoxynitro-L-arginyl-L-phenylalanine (0.5 g.) was hydrogenated in methanol containing 10% of acetic acid in the manner described. The dipeptide acetate precipitated during the course of the hydrogenation and was redissolved by the addition of small quantities of glacial acetic acid. The material crystallized on removal of the solvent, and was recrystallized from aqueous ethanol; yield 0.24 g. (63%); m.p. 172–173°; $[\alpha]^{28}D$ +29.5° (*c* 1.81, in water).

Anal. Calcd. for $C_{17}H_{27}O_5N_6$: C, 53.5; H, 7.1; N, 18.4. Found: C, 53.6; H, 7.4; N, 18.6.

L-Arginyl-L-tyrosine Acetate.—Carbobenzoxynitro-L-arginyl-L-tyrosine (0.8 g.) was hydrogenated in methanol-acetic acid as described. The sirup obtained on evaporation of the solvents crystallized on standing and the peptide was recrystallized from aqueous ethanol; yield 0.5 g. (81%); m.p. 157–158°; $[\alpha]^{32}D$ +33.3° (*c* 1.53, in water).

Anal. Calcd. for $C_{17}H_{27}O_6N_6$: C, 51.4; H, 6.9; N, 17.6. Found: C, 51.3; H, 7.1; N, 18.0.

L-Arginyl-L-tryptophan Acetate.—Carbobenzoxynitro-L-arginyl-L-tryptophan (0.75 g.) was hydrogenated in methanol-acetic acid for 12 hours. The sirup obtained on evaporation of the solvents failed to crystallize. The dipeptide was obtained in the form of a hygroscopic powder by repeated precipitation from methanol with ether; yield 0.30 g. (52%); $[\alpha]^{28}D$ +5.1° (*c* 4.94, in water).

Anal. Calcd. for $C_{19}H_{28}O_5N_6$: N, 20.0. Found: N, 19.1.

L-Arginyl-L-glutamic Acid.—Carbobenzoxynitro-L-arginyl-L-glutamic acid (0.8 g.) was hydrogenated in methanol-acetic acid for 12 hours. The crystalline residue which remained upon evaporation of the solution, was recrystallized from aqueous ethanol; yield 0.48 g. (96%); m.p. 251–252° dec. (lit.[7] m.p. 205–210°); $[\alpha]^{25}D$ +24.8° (*c* 1.90, in water).

Anal. Calcd. for $C_{11}H_{21}O_5N_5$: C, 43.6; H, 7.0; N, 23.1. Found: C, 43.8; H, 7.0; N, 23.2.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF PENNSYLVANIA]

Metabolite Analogs. V. Preparation of Some Substituted Pyrazines and Imidazo[b]pyrazines

BY FRANK L. MUEHLMANN AND ALLAN R. DAY

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A number of pyrazines and imidazo[b]pyrazines have been prepared as potential metabolic antagonists of essential pyrimidines and purines.

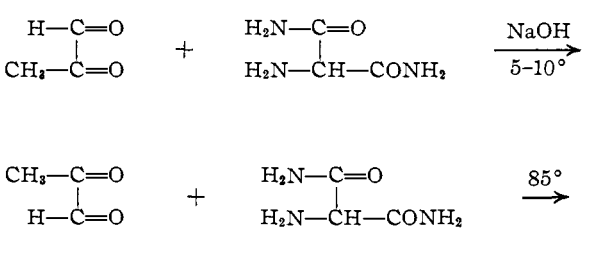
A number of imidazo[b]pyrazines have been prepared from the corresponding 2,3-diaminopyrazines. The preparation of the imidazo[*b*]pyrazine ring system was first reported in 1952.[1] In general the first step involves the condensation of a suitable 1,2-dicarbonyl compound with aminomalonamide to form the corresponding 2-hydroxy-3-carboxamidopyrazine. Although the conditions for these reactions had been reported previously,[2] it has been found in the course of the present study that reproducible results could not be obtained. Consequently a careful examination of the reaction conditions was made.

It has been found that the procedure of Jones for the condensation of diacetyl with aminomalonamide gave the best yields of 2-hydroxy-3-carboxamido-5,6-dimethylpyrazine (75%) when the sodium hydroxide was omitted and the reaction was carried out at 85°. The condensation of glyoxal with aminomalonamide was also modified considerably. The use of glyoxal bisulfite in place of glyoxal has been reported[2] but neither experimental conditions nor yields were given. In our laboratory the best results were obtained when an aqueous

(1) E. Schipper and A. R. Day, THIS JOURNAL, **74**, 350 (1952).
(2) R. G. Jones, *ibid.*, **71**, 78 (1949).

solution of glyoxal bisulfite was heated at 80° with aminomalonamide giving 80–85% yields of 2-hydroxy-3-carboxamidopyrazine.

With commercial methylglyoxal and aminomalonamide in the presence of sodium hydroxide at low temperatures, 25–30% yields of 2-hydroxy-3-carboxamido-5-methylpyrazine have been reported.[2] In the present investigation, it has been found that the bisulfite method used for glyoxal may be applied to commercial methylglyoxal also although the yields are lower (45%). The methyl-2-hydroxy-3-carboxamidopyrazine obtained by this method melted at 227° whereas the one obtained by Jones melted at 244°. The corresponding acids melted at 205° and 155–157°, respectively. These data along with the analytical results indicate that the two carboxamides and the corresponding acids are isomeric compounds. The free acid obtained in the present study appears to be identical with the one obtained from 7-methyllumazine,[2] namely, 2-hydroxy-6-methylpyrazine-3-carboxylic acid.



Ethyl pyruvate also has been condensed with aminomalonamide to give 2,5(6)-dihydroxy-3-carboxamido-5(6)-methylpyrazine. No attempt has been made as yet to ascertain the actual positions of the hydroxyl and methyl groups.

The second step in the synthesis of the imidazopyrazines was the conversion of the 2-hydroxy-3-carboxamidopyrazines to the corresponding 2-hydroxy-3-amino compounds by means of the Hofmann reaction. In general, good yields were obtained in these reactions.

The third step, the conversion of the 2-hydroxy-3-aminopyrazines to the corresponding 2-chloro derivatives, proved to be the most difficult. It has been reported that 2-hydroxy-3-aminopyrazine gave a 67% yield of 2-chloro-3-aminopyrazine when treated with phosphorus oxychloride.[3] In the present investigation, this reaction was carried out exactly as described but the yield was never greater than 10% and usually smaller. After many variations, it was found that yields of 25–38% could be obtained by heating the reactants under pressure. It appears to be necessary to prevent the escape of hydrogen chloride to get even fair yields of the chloro compound.

The preparation of 2,3-diaminopyrazine was effected by heating the chloro compound with ammonia under pressure in the presence of copper as a catalyst.[3] The yields were fairly good under carefully controlled conditions.

Finally the diamine was converted to the corresponding imidazopyrazines by heating with ethyl orthoformate, acetic anhydride, urea and glycolic acid, respectively.

Both the substituted pyrazines and imidazo[b]-pyrazines are being screened for physiological activity. These results will be reported elsewhere.

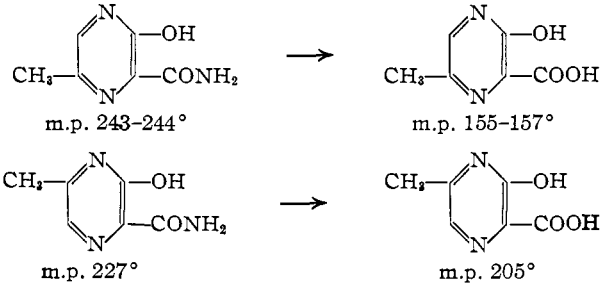
(3) F. G. McDonald and R. C. Ellingson, THIS JOURNAL, **69**, 1034 (1947).

Experimental

Preparation of 2-Hydroxy-3-carboxamido-5,6-dimethylpyrazine.—The procedure of Jones[2] was used except that sodium hydroxide was not used and the reaction was carried out at 85°, yields 70–80%.

Preparation of 2-Hydroxy-3-carboxamidopyrazine.—Glyoxal sodium bisulfite hemihydrate[4] (170 g.) was dissolved in 400 ml. of water. To this was added 58.5 g. (0.5 mole) of aminomalonamide[1] and the solution stirred at 80° for 3 hours. Sodium acetate (170 g.) was now dissolved and the resulting solution cooled to 20°. Hydrogen peroxide (130 ml. of 30%) was added gradually with stirring and the temperature of the solution was not allowed to go above 60–65°. After cooling overnight, the yellow crystals were removed and washed with a small amount of cold water, yield 85%, m.p. 268° dec. The product was recrystallized from water and obtained as pale yellow crystals, m.p. 270° dec.

Preparation of 2-Hydroxy-3-carboxamido-6-methylpyrazine.—Sodium bisulfite (30 g.) was added to 36 ml. of 40% methylglyoxal solution and to this solution was added gradually 100 ml. of water containing 1 g. of sodium hydroxide. The solution was heated to 80° for 1 hour, 23.4 g. (0.2 mole) of aminomalonamide added and the temperature of the solution maintained at 80° for 2 more hours with stirring. Sodium acetate (40 g.) was added, the solution cooled to 20° and 30 ml. of 30% hydrogen peroxide added gradually with stirring. The temperature was kept at 60–65°. After cooling overnight, the yellow product was removed and washed with cold water. It was purified by recrystallization from 75% ethyl alcohol with the aid of decolorizing carbon, yield 45%, light yellow crystals, m.p. 227°.



Anal. Calcd. for $C_6H_7O_2N_3$: C, 47.10; H, 4.58; N, 27.45. Found: C, 47.20; H, 4.66; N, 27.44.

Preparation of 2-Hydroxy-6-methylpyrazine-3-carboxylic Acid.—2-Hydroxy-3-carboxamido-6-methylpyrazine (4 g.) was added to 20 ml. of 5 *N* NaOH and heated on the steam-bath for 20 hours. The solution was neutralized with concentrated hydrochloric acid and cooled overnight. The product was removed, washed with a little cold water and dried, yield 82%, m.p. 184–185° dec.[5] After recrystallization from water, with the aid of decolorizing carbon, colorless crystals were obtained, m.p. 205° dec.

Anal. Calcd. for $C_6H_6O_3N_2$: C, 46.75; H, 3.92; N, 18.18. Found: C, 46.78; H, 3.81; N, 18.27.

Preparation of 2,5(6)-Dihydroxy-3-carboxamido-5(6)-methylpyrazine.—Sodium (6 g.) was dissolved in 100 ml. of dry methanol. This solution was added with stirring to a suspension of 23.4 g. (0.2 mole) of aminomalonamide in 200 ml. of dry methanol. Thirty ml. of ethyl pyruvate, dissolved in 100 ml. of dry methanol, was added dropwise and the mixture gradually brought to boiling on the steam-bath. After the addition of the ethyl pyruvate, heating and stirring were continued for 2 more hours. The suspended material changed from yellow to red during this period. After cooling overnight, the product was removed and washed with a small amount of methanol. It was then dissolved in 200 ml.

(4) A. R. Ronzio and T. D. Waugh, *Org. Syntheses*, **24**, 61 (1944).

(5) This corresponds to the m.p. reported by Jones.

of water with the aid of a small amount of sodium hydroxide, made slightly acid with concentrated hydrochloric acid, cooled to 10°, and the product removed by filtration, yield 25%. Final purification was effected by recrystallization from a large volume of water (500 ml. per gram) with the aid of decolorizing carbon, light yellow crystals, darkening at 240°, m.p. about 360°.

Anal. Calcd. for $C_6H_7O_3N_3$: C, 42.60; H, 4.17; N, 24.85. Found: C, 42.50; H, 4.19; N, 24.90.

Preparation of 2-Hydroxy-3-aminopyrazine.—Potassium hydroxide (132 g.) was dissolved in 1000 ml. of water. The solution was cooled to 5° and 21.4 ml. of bromine added gradually with stirring. Ice-water (500 ml.) was added, then 55.6 g. (0.4 mole) of 2-hydroxy-3-carboxamidopyrazine added all at once with stirring. The temperature of the solution was raised to 85° and maintained at this point for 1.5 hours. The solution was then cooled to 20°, acidified with concentrated hydrochloric acid, then made alkaline with concentrated ammonium hydroxide and cooled overnight. The product was removed, washed with cold water and dried, yield 97%. It was recrystallized from water, m.p. 300–301° dec.

Preparation of 2-Hydroxy-3-amino-6-methylpyrazine.—The above procedure was applied to 2-hydroxy-3-carboxamido-6-methylpyrazine, yield 94%. The product was purified by recrystallization from water (500 ml. per gram) with the aid of decolorizing carbon, colorless crystals, m.p. 335–337° dec.

Anal. Calcd. for $C_5H_7ON_3$: C, 47.99; H, 5.64; N, 33.58. Found: C, 48.06; H, 5.57; N, 33.50.

Preparation of 2-Chloro-3-aminopyrazine.—Powdered 2-hydroxy-3-aminopyrazine (2.2 g., 0.02 mole) was placed in a 200-ml. pressure flask. Five ml. of ice-cold phosphorus oxychloride was added and then 0.5 ml. of ice-water was added by allowing it to run down the side of the flask from a pipet and the flask tightly stoppered at once. After mixing, the reaction mixture was stirred with a magnetic stirrer and heated to 115° for 6 hours. The flask was cooled to 10° and the clamp which held in the rubber stopper cautiously opened.

Small pieces of ice (20–30 g.) were added and the resulting solution transferred to a flask. Usually 10 runs were made before working up the solution. The combined solutions were heated with decolorizing carbon and filtered with the aid of Celite. The filtrate was cooled to 10° and made slightly alkaline with concentrated ammonium hydroxide. After cooling overnight, the product was removed, washed with water and dried, weight 19.5 g. This proved to be a mixture of 2-chloro-3-aminopyrazine and starting material. The chloro compound was removed by extraction with chloroform in a Soxhlet extractor. The chloroform on evaporation gave light yellow crystals, yields 27–38%, m.p. 169°. The unreacted 2-hydroxy-3-aminopyrazine was used over again.

Preparation of 2,3-Diaminopyrazine.—Powdered 2-chloro-3-aminopyrazine (12.95 g., 0.1 mole) was placed in a pressure tube and a small amount of freshly prepared copper powder added. Forty ml. of concentrated ammonium hydroxide was added, the glass tube sealed and heated at 135° for 24 hours. After cooling, the tube was opened and heated in hot water to redissolve any solid that may have separated. The solution was transferred to an extractor and the diamine extracted with ethyl acetate. Decolorizing carbon was added to the ethyl acetate solution, the resulting mixture refluxed for 30 minutes and filtered. The filtrate was concentrated to about 30 ml. and cooled overnight, light brown crystals, yields 50–59%, m.p. 203°.

Preparation of Imidazo[b]pyrazine.—Two grams of 2,3-diaminopyrazine was heated in a test-tube with 10 ml. of ethyl orthoformate at 140–145° for 2 hours. At this time the volume was reduced to about 5 ml. This was transferred to a small beaker, 20 ml. of 10% sodium hydroxide and decolorizing carbon added and the mixture heated on a steam-bath for 10 minutes. After cooling, the filtrate was acidified with acetic acid and cooled overnight. The product was removed by filtration and dried, yield 78%. It was further purified by recrystallization from water, m.p. 257°.

Anal. Calcd. for $C_6H_4N_4$: C, 50.00; H, 3.35; N, 46.65. Found: C, 50.00; H, 3.29; N, 46.69.

Preparation of 2-Methylimidazo[b]pyrazine.—Two grams of 2,3-diaminopyrazine in 10 ml. of acetic anhydride was heated for one hour at 140°. Most of the excess acetic anhydride was removed by distillation and the residue dissolved in 20 ml. of hot 10% sodium hydroxide solution. This solution was treated with decolorizing carbon and filtered while hot. The filtrate was acidified with acetic acid and cooled overnight. The product was removed and washed with ice-water, yield 82%. It was recrystallized from hot water (200 ml. per g.), colorless crystals, m.p. 370° dec.

Anal. Calcd. for $C_6H_6N_4$: C, 53.72; H, 4.51; N, 41.77. Found: C, 53.84; H, 4.46; N, 41.78.

Preparation of 2-Hydroxyimidazo[b]pyrazine.—Two grams of 2,3-diaminopyrazine and 2 g. of urea were heated at 160° for 2 hours. The mixture was then dissolved in 50 ml. of hot 2% sodium hydroxide solution, treated with decolorizing carbon and filtered while hot. The filtrate was acidified with acetic acid and cooled overnight. The product was removed, washed with cold water and dried, yield 90%. It was recrystallized from ethyl alcohol, pale yellow crystals, m.p. 336°.

Anal. Calcd. for $C_6H_4ON_4$: C, 44.12; H, 2.96; N, 41.17. Found: C, 43.98; H, 2.84; N, 41.04.

Preparation of 2-Hydroxymethylimidazo[b]pyrazine.—Two grams of 2,3-diaminopyrazine and 3 g. of glycolic acid were heated at 140–145° for 2 hours. The mixture was then dissolved in 100 ml. of hot water, treated with decolorizing carbon, filtered and dried, yield 73%. It was recrystallized from water, m.p. 309° dec.

Anal. Calcd. for $C_6H_6ON_4$: C, 48.00; H, 4.03; N, 37.32. Found: C, 48.08; H, 4.10; N, 37.29.

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